ANALYSIS USING MICROFLUIDIC PARTITIONING DEVICES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. provisional application No. 60/687,010, filed Jun. 2, 2005, the entire contents of which are herein incorporated by reference.

FIELD OF THE INVENTION

[0002] The invention relates to methods, reagents and devices for detecting and characterizing nucleic acids, cells, and other biological samples.

BACKGROUND

[0003] A variety of nucleic acid amplification assays and immunological assays are used for analysis of cells and nucleic acids. These assays can be used to detect or characterize nucleic acid sequences associated with particular diseases or genetic disorders, for genotyping, for gene expression analyses, to detect and identify pathogens such as viruses, bacteria and fungi), for paternity and forensic identification, and for many other purposes. However, in some applications the efficiency and sensitivity of these assays is reduced, which may render the assays useless or at minimum require that additional manipulations and/or significant amounts of expensive reagents be used. For example, when a cell or molecule to be analyzed is from a sample with a large excess of non-target cells or molecules (e.g., as in genetic or phenotypic analysis of a rare cell in a background of other cells) conventional assay methods are inadequate. Similarly, when a number of different targets must be detected in a single sample, conventional approaches (e.g., multiplex PCR) are expensive, inefficient or not sufficiently sensitive. Thus, new methods, reagents and devices for detection and characterization of nucleic acids, cells, and other biological molecules will find broad application in biomedicine and other fields.

BRIEF SUMMARY

[0004] The invention relates to methods, reagents and devices for detection and characterization of nucleic acids, cells, and other biological samples. In one aspect, the invention provides an assay method including the following steps (a) partitioning a sample into a plurality of sub-samples, where said sample comprises a plurality of nucleic acid molecules, and where at least two sub-samples comprise at least one nucleic acid molecule; (b) providing sufficient reagents in each sub-sample to amplify a target sequence or sequences; (c) amplifying the target sequence(s) in the sub-sample(s) containing target sequence(s) thereby producing amplicons in the sub-sample; (d) distributing the amplicons into a plurality of aliquots; and, (e) for each aliquot, determining a property of amplicons in the aliquot.

[0005] In a related aspect, the invention provides an assay method including the following steps (a) partitioning a sample into a plurality of sub-samples, where said sample comprises a plurality of nucleic acid molecules, and where at least two sub-samples comprise at least one nucleic acid molecule; (b) providing sufficient reagents in each sub-sample to amplify at least two different target sequences; (c) amplifying target sequence(s) in at least two sub-sample(s) thereby producing amplicons in the sub-sample(s); (d) combining the amplicons from said at least two sub-samples to create an amplicon pool; (d) dividing the amplicon pool into a plurality of aliquots; and, (e) for each aliquot, determining a property of amplicons in the aliquot. In one embodiment, the sample is partitioned into at least 10^4 subsamples. In one embodiment, each subsample has a volume of less than one nanoliter. In one embodiment, the nucleic acid molecules comprise DNA and/or mRNA. In one embodiment, the amplification is by PCR or RT-PCR. In one embodiment, sufficient reagents are provided to amplify at least 10, 20, or 50 different target sequences, if present. In one embodiment, the amplicon pool is divided into at least 10, 20, 50 or 100 aliquiots. In one embodiment, the sample contains a plurality of cells having nucleic acid molecules, and partitioning the sample involves partitioning intact cells into a plurality of sub-samples. In one embodiment, the sample contains only one cell.

[0006] In another aspect, the invention provides an assay method including the following steps (a) partitioning a sample comprising a plurality of separable cells into at least 1000 separate reaction chambers in a massively partitioning device (MPD), where after partitioning at least two reaction chambers each comprise exactly one cell; (b) providing in each reaction chamber one or more reagents for determining a property or properties of a cell, where the same reagents are provided in each chamber; and (c) determining at least two different properties of a single cell in a chamber and/or determining at least one property for at least two different cells in different chambers. In one embodiment, at least 99% of the reaction chambers contain zero or one cell. In one embodiment, the cells are bacterial cells. In one embodiment, the reagents include reagents for nucleic acid amplification. In one embodiment, at least one property is the presence or absence in the cell of a nucleic acid having a specified sequence. In one embodiment, at least one property is other than the presence or absence in the cell of a nucleic acid having a specified sequence.

[0007] In another aspect, the invention provides a method for amplification and detection of multiple target DNA sequences in a sample, including the following steps: (a) providing a sample containing (i) multiple target DNA sequences, (ii) a primer pair corresponding to each of said multiple target DNA sequences, each pair consisting of a first primer comprising U_1, B_1 and F domains in the order 5'-U_1-B_1-F-3' and a second primer comprising U_2 and R domains in the order 5'-U_2-R-3', where each pair of F and R primers is capable of annealing specifically to a different target DNA sequence under stringent annealing conditions; (iii) a universal primer pair capable of amplifying a double stranded DNA molecule with the structure

5'-U_1--U_2-3'

3-U_1'---U_2-3'

where U_1' is the sequence complementary to U_1 and U_2' is the sequence complementary to U_2; (b) subjecting the sample to multiple cycles of melting, reannealing, and DNA synthesis thereby producing amplicons for each of said multiple target DNA sequences, and (c) detecting the amplicons using a probe that anneals to sequence of the amplicon having the sequence of the B_1 domain or its complement. In one embodiment, the sample also contains a second set of multiple target sequences, a primer pair corresponding to to each of the target sequences in the second set, each pair consisting of a first primer comprising U_1, B_2 and F domains in the order 5'-U_1-